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(54) Title: PHARMACEUTICAL PREPARATION AND A PROCESS FOR MAKING SAME

(57) Abstract

The invention provides for both a pharmaceutical preparation and a method for inhibiting *in vivo* the reverse transcriptase enzyme and the replication of human immunodefiency virus (HIV). In one embodiment the pharmaceutical preparation is a complex of dextran, modified dextran, dextran sulphate or polysaccharides and 3'-azido-2',3'-dideoxythymidine (AZT) which may be administered via different routes in appropriate dosage forms, dosage quantities and dosage regimens to patients suffering from a viral disease such as AIDS and its related disorders. This complex represents a novel structure which functions as a structural unit which combines the known additive and synergistic properties of dextran or dextran sulphate with AZT and at the same time appears to ameliorate the toxic effects of AZT.

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PHARMACEUTICAL PREPARATION AND A PROCESS FOR MAKING SAME

This invention relates to a pharmaceutical preparation and method for inhibiting reverse transcriptase enzyme and the replication of a family of viruses known as human immunodeficiency virus (HIV) by the use of a novel complex of dextran, modified dextran, dextran sulphate, dextrin, cellulose or other polysaccharides with AZT.

BACKGROUND TO THE INVENTION

Certain retrovirus infections have been known to depress immune functions in animals. In recent years, it has been discovered that a family of T-lymphotropic retroviruses causes T-cell proliferation leukaemia, helper T-cell depletion and immunosuppression in humans infected by these viruses. These viruses have become known as the HTLV family of retroviruses. A group of these viruses designated as HTLV-III has been isolated from patients with Acquired Immune Deficiency Syndrome (AIDS) and has become considered to be responsible for the development of this condition in humans. These are also known as HIV, particularly HIV-1 and HIV-2.

Since the epidemic was recognized in 1981, a rapidly increasing number of cases of AIDS have been diagnosed in the United States. Significant progress has resulted from research in selected areas including identification of the populations at risk for AIDS, the method of transmission of the causative agent, isolation and characterization of the virus that causes AIDS and development of a serologic test that identifies most infected individuals.

On the other hand little progress has been made toward effective treatment of AIDS. Azidothymidine (AZT), a drug that inhibits reverse transcriptase was expected to prolong the lives of patients with AIDS but this is now in doubt. Many patients who receive AZT have temporary increases in the number of circulating helper (CD4+) T-lymphocytes. However, the drug has significant adverse affects and HIV has been isolated from the blood of patients even while they are under treatment with AZT.

Although zidovudine (3'-azido-2', 3'-dideoxythymidine, AZT, azidothymidine, Retrovir) is the FDA-approved drug, it has undesirable toxicity in the host (e.g. myelosuppression, neuropathy).

Progressive immunological and central nervous system disease is coupled to virus replication. The general observation is that the more the virus replicates, the more serious the disease symptoms. Prolonged virus replication results in near total destruction of immune function. This observation leads to the conclusion that the central goal of AIDS therapy is to control and hopefully eliminate virus replication.

SUMMARY OF THE INVENTION

The invention provides for a pharmaceutical preparation for inhibiting *in vivo* the reverse transcriptase enzyme and the replication of viruses, which comprises a complex of Dextran, Modified Dextran, Dextran Sulphate, Dextrin, Cellulose or other Polysaccharides and AZT in appropriate pharmaceutical dosage form. The invention also provides a method for inhibiting *in vivo* the replication of HIV viruses comprising the different routes of administration of the aforesaid complexes in a pharmaceutically appropriate dosage form, regimen and quantity.

DESCRIPTION OF THE INVENTION

Those skilled in the art will be aware of pharmaceutically appropriate dosage forms for the complex of Dextran, Modified Dextran, Dextran Sulphate, Dextrin, Cellulose or other Polysaccharides and AZT as well as the manner in which a suitable dosage quantity, regimen and routes of administration may be derived in respect of a particular patient.

Modified Dextran may be substituted, oxidised, cationic, anionic, spacer or activated Dextran.

The demonstrated absorption qualities of Dextran, Modified Dextran, Dextran Sulphate, Dextrin, Cellulose and other Polysaccharides with AZT will bring these complexes into the endothelium system with a consequently improved effect in the treatment of viral diseases.

As Dextran, Modified Dextran, Dextran Sulphate, Dextrin, Cellulose and other Polysaccharides couples with AZT the following advantages will be exhibited.

1) Above mentioned complexes as macromolecular compounds have excellent metabolic stability, resulting in a more effective treatment.
2) Because the complexes are large molecules and consist of polysaccharides, they are easy to be received and combined by receptors of the cell which consist of polysaccharide-protein.
3) Dextran and certain derivatives such as the sulphate are useful not only as a "transfer weapon" but also as an immunologically active material which has now been used in the treatment study of AIDS.
4) The complex provides an effective weapon which will be able to attack and kill the harmful cells of several

severe diseases including AIDS and Carcinoma.

5) A Dextran-AZT complex is a carrier or stabilizer frequently resulting in decreased drug toxicity, after biodistribution, and mostly increases therapeutic efficacy.

6) A Dextran Sulphate-AZT complex increases the bioadhesiveness with increase in polyanionic character as it cannot be captured by the first pass effect and also during circulation.

7) Macromolecules like complexes of Dextran, Modified Dextran, Dextran Sulphate, Dextrin, Cellulose or other Polysaccharides with AZT remain for prolonged periods of time in contact with cell receptors and appear to inhibit contact of certain viruses with cell receptors.

8) Immunogenecity of drugs could be decreased by these complexes.

9) Complexes of AZT with Dextran, Modified Dextran, Dextran Sulphate, Dextrin, Cellulose or other Polysaccharides will be useful as macromolecular pro-drugs in drug delivery systems and suitable as nontoxic carriers for more effective drugs for the treatment of viral diseases.

The Dextran complexed with AZT may have a molecular weight within the range of 4,000 to 1,000,000. The Dextran Sulphate complexed with AZT may have a molecular weight within the range of 8,000 to 1,000,000. Examples of polysaccharides which may be complexed with AZT are dextrin, cyclodextrin, cellulose and cellulose sulphate. A useful complex of AZT and a polysaccharide will be one exhibiting a slow release mechanism.

The aforesaid complexes may include a pharmaceutically acceptable carrier or diluent.

The pharmaceutical preparations the subject of this invention are not only useful in the treatment of AIDS and

other viral diseases, but are also useful in the treatment of AIDS-related complex (A.R.C.).

The present invention also contemplates a pharmaceutical preparation useful for the treatment of AIDS comprising a complex of a known anti-AIDS agent with (a) Dextran (b) Modified Dextran (c) Dextran Sulphate (d) Dextrin, (e) Cellulose or, (f) a polysaccharide exhibiting a slow release mechanism.

Methods for production of the AZT complexes with Dextran

Method A:

1) 1 gram of Dextran powder (MW 40,000) dissolved in 10 ml of DMF:H20 (1:1) mixture by warming
2) PH adjusted to 8.0 with 16% sodium carbonate solution
3) 0.5 gram of cyanogen bromide in 5 ml of DMF was dissolved
4) 0.75 ml of triethylamine in 5 ml of DMF was mixed
5) After cooling Dextran solution added 0.5 ml of each solution #3 and 4
6) After 2 minutes 1.0 gram of 6-amino caproic acid was added and stirred overnight at room temperature
7) Purified sample with precipitation with methanol
8) 100 mg of above Modified Dextran dissolved in 7.5 ml of DMSO
9) Added 13.5 mg of AZT
10) 9.0 mg of 4-dimethyl aminopyridine was added
11) 13.0 mg of N, N-dicyclohexyl carbodiimide was added and the reaction mixture was stirred at room temperature for 16 hours at anhydrous conditions
12) Purified by dialysis, filtered with 0.45 micron filter paper and dried.

The resulting product was a stable complex of dextran

with AZT.

Method B:

1) 1.0 gm of Dextran (MW 40,000) powder dissolved in water (5 ml)
2) NAOH (40%, 7.5 ml) added
3) Chloroacetic acid (5.4 g) added and reacted at room temperature for 16 hours
4) Purified sample with precipitation and dried
5) 100 mg of above Modified Dextran dissolved in 7.5 ml of DMSO
6) 13.5 mg of AZT was added
7) 9.0 mg of 4-dimethyl amino pyridine was added
8) 13.0 mg of N, N-dicyclohexyl carbodiimide was added and reaction mixture was stirred at room temperature for 16 hours at anhydrous conditions.
9) Purified by dialysis, filtered with 0.45 micron filter paper and dried.

Product-complex or conjugate of dextran - AZT.

Method C:

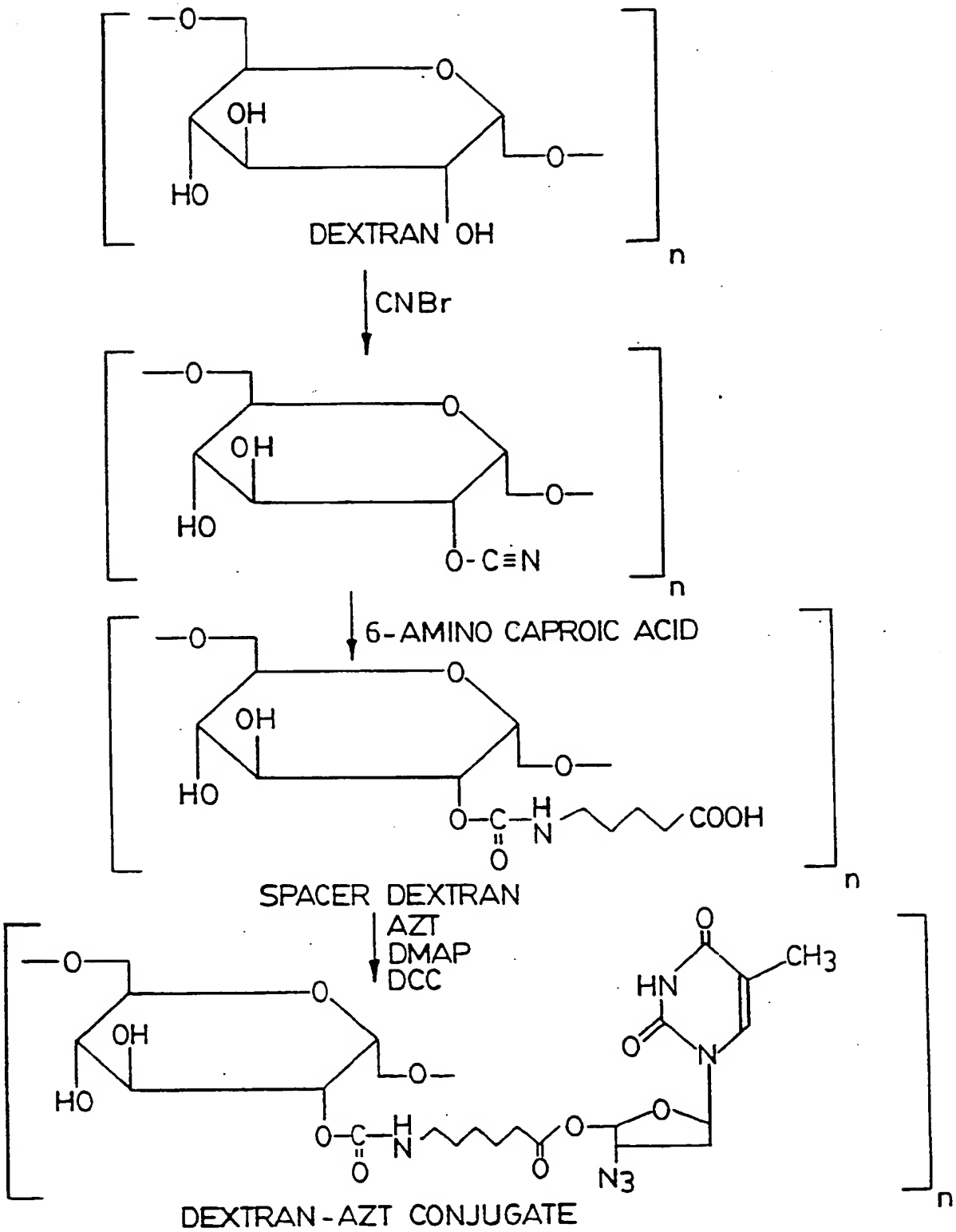
1) 1.0 gm of Dextran (MW 40,000) powder dissolved in 10 ml of DMF:H20 (1:1) mixture
2) Adjusted PH to 8.0 with 16% sodium carbonate solution
3) Cooled it below freezing temperature
4) 0.5 gm of cyanogen bromide was dissolved in 5 ml of DMF
5) 0.75 ml of triethylamine was mixed with 5.0 ml of DMF
6) After cooling Dextran solution, 0.5 ml of each solution #4 and 5 were added
7) After 5 minutes 0.8 gm of N-acetyl cysteine was added
8) PH of solution was adjusted to 9.0 with 16% sodium carbonate solution

9) Stirred for 24 hours at room temperature
10) Purified sample with precipitation and dried
11) 100 mg of above Modified Dextran was dissolved in 10 ml of DMSO
12) 13.5 mg of AZT was added
13) 9.0 mg of 4-dimethyl amino pyridine was added
14) 13.0 mg of N, N-dicyclohexyl carbodiimide was added and reaction mixture was stirred at room temperature for 16 hours
15) Purified by dialysis, filtered with 0.45 micron filter paper and dried.

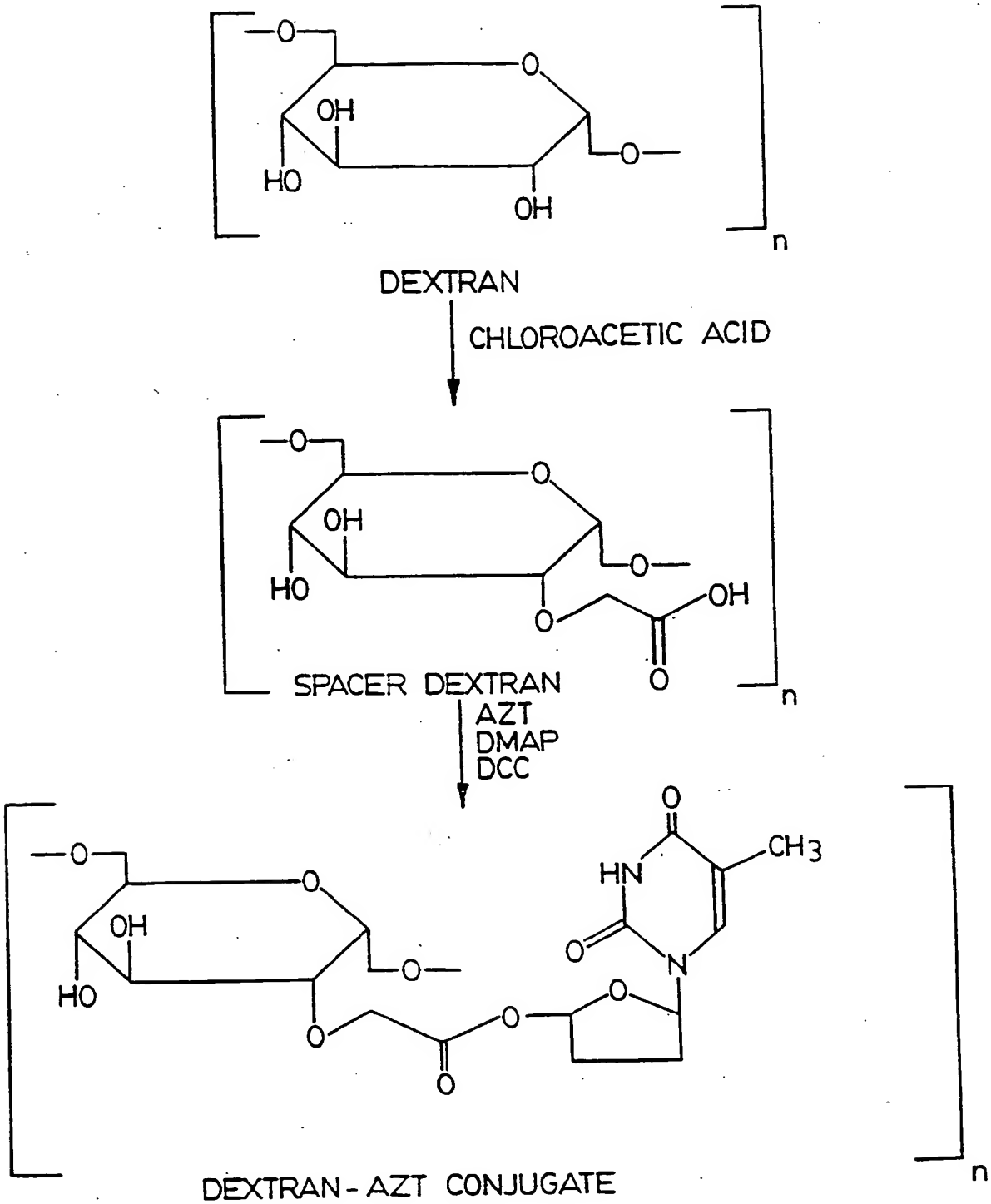
The resulting product was a complex or conjugate of dextran and AZT.

Structural diagrams relating to the aforedescribed methods A, B and C are set out hereinafter.

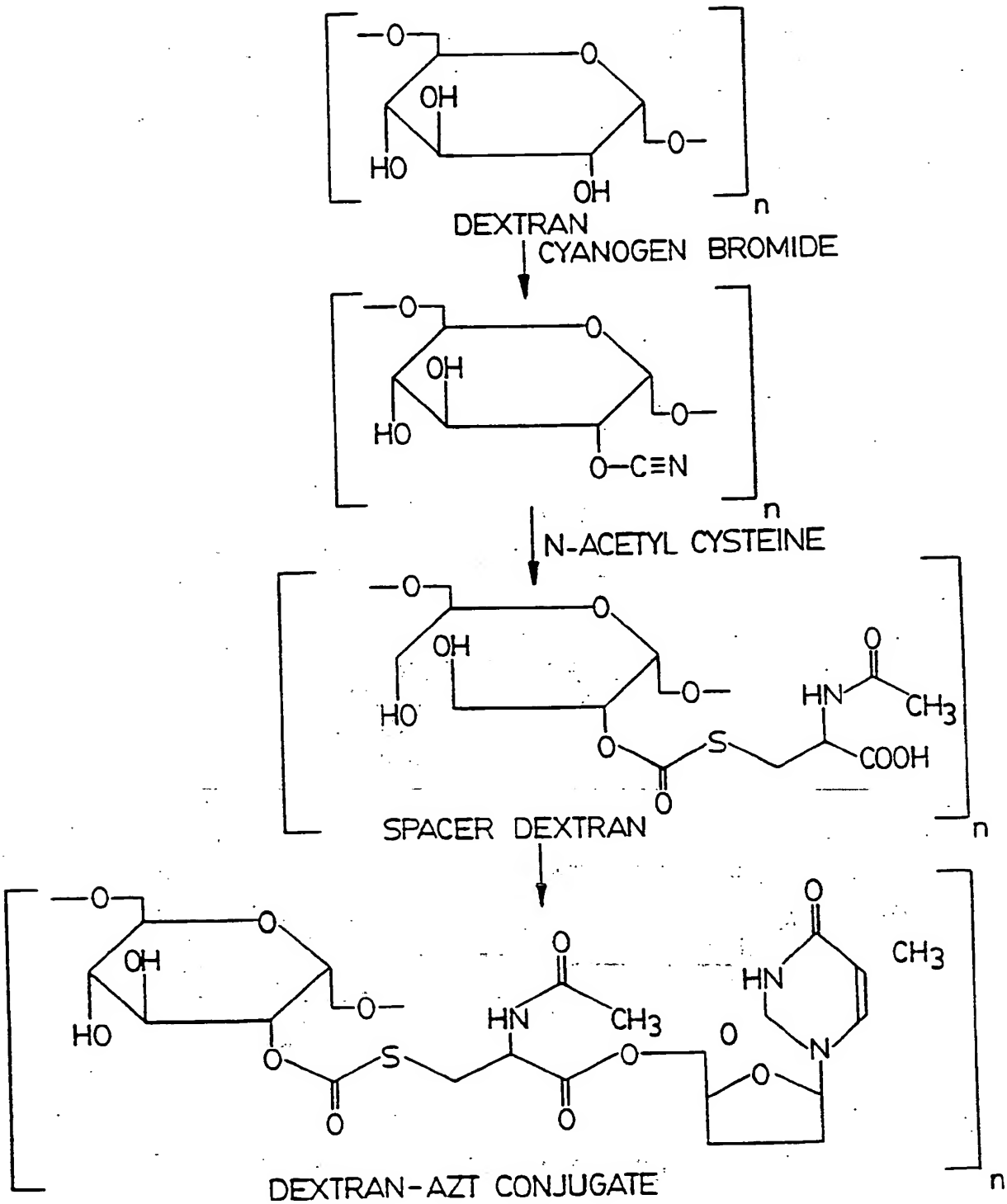
METHOD A


DEXTRAN OH
CNBr
6-AMINO CAPROIC ACID
SPACER DEXTRAN
AZT
DMAP
DCC
DEXTRAN-AZT CONJUGATE

METHOD B


DEXTRAN
CHLOROACETIC ACID
SPACER DEXTRAN
AZT
DMAP
DCC
DEXTRAN - AZT CONJUGATE

METHOD C


DEXTRAN
CYANOGEN BROMIDE
N-ACETYL CYSTEINE
SPACER DEXTRAN
DEXTRAN-AZT CONJUGATE

Methods for AZT conjugation with dextran sulphate

Method A

1. Dissolve (350 mg) Dextran Sulphate (MW 8000 to 1 Million or more) in DMSO (10 ml) by warming
2. Add 27.0 mg AZT and 5 ml pyrodine and 2 ml DMSO
3. Add 130 mg of Dicyclohexyl carbodiimide
4. Keep solution stirring at 50° C for 24 hours and then add about 5 ml water
5. Dialyzed against water, filter with 0.2 micron filter
6. Freeze dry

Method B

1. Mix 25.0 mg of Diisothiocyanate Distilbene sulfonic acid Na Salt hydrate and 13.0 mg of AZT in 10 ml toluene
2. Add 10 mg of Dimethyl amino pyridine
3. Reaction mixture stirred for 6 hours at 50° C, lower the temperature to 37° C and continue reaction at 37° C for 18 hours
4. Evaporate toluene (Yellow Powder)
5. Dissolve above yellow powder in 20 ml solution of 1:1 DMSO & Pyridine mixture
6. Add 250.0 mg of Dextran Sulphate (MW of 1 million or more) in above solution
7. Reaction stirring at 37° C for 24 hours
8. Add 5 ml water and dialyse the solution against water
9. Filter through 0.2 micron filter
10. Freeze dry

The aforesaid methods relating to Dextran and Dextran Sulphate may also be employed to conjugate other polysaccharides including dextrin, cyclodextrin, cellulose, cellulose sulphate and Modified Dextran with AZT.

Test results

DRUG	CONCENTRATION (ug/ml)	# OF SYNCYTIA	PER WELL
1. AD 042793	100	0	0
Dextran-AZT	10	40	42
complex	1	53	55
	0.1	52	49
2. AZT standard	100	0	0
	10	0	0
	1	0	0
	0.1	10	8
3. AZT 041493	100	13	12
Dextran-AZT	10	48	50
complex	1	54	50
(second batch)	0.1	52	51

CONTROLS	CONCENTRATION (ug)	# OF SYNCYTIA	PER WELL
CELLS (NO VIRUS/ NO DRUG)	0	0	0
(VIRUS/NO DRUG)	54	49	53
AZT POSITIVE CONTROL	10.0	0	0
	1.0	0	0
	0.1	10	11

Further Examples

A. Dextran + Succinic Anhydride + AZT Conjugation

General Method

1. To a solution of 20 - 200 mg of AZT, 2 - 20 mg of 4-dimethyl amino pyridine (DMAP) and 10 - 100 mg of anhydrous sodium sulfate (NA2So4) in 1 - 10 ml of pyridine add 10 - 100 mg of succinic anhydride.

2. Stir the resulting slurry under closed top at room temperature to 40°C for 1 - 24 hours.

3. Add 50 - 500 mg of dextran solution in 5 - 25 ml of dimethyl sulfoxide (DMSO).

4. Add 5 - 50 mg of each 4-dimethylamino pyridine (DMAP) and dicyclohexyl carbodiimide (DCC), stir the reaction mixture for 12 - 96 hours.

5. Add 20 ml water, cool the solution, filter.

6. Dialyse the solution against water (changing water 3 times a day) for 3 days.

7. Filter the solution and extract it with chloroform (4 times) to remove any free organic material (viz DMAP, DCC, AZT).

8. Freeze dry the aqueous solution.

Preferred Method

1. To a solution of 54 mg of AZT, 6 mg of 4-dimethyl amino pyridine (DMAP) and 20 mg of anhydrous sodium sulfate

(NA2So4) in 2 ml of pyridine add 25 mg of succinic anhydride.

2. Stir the resulting slurry under closed top at room temperature for 3 hours.

3. Add 100 mg of dextran solution in 10 ml of dimethyl sulfoxide (DMSO).

4. Add 20 mg of each 4-dimethylamino pyridine (DMAP) and dicyclohexyl carbodiimide (DCC), stir the reaction mixture for 72 hours.

5. Add 20 ml water, cool the solution, filter.

6. Dialyse the solution against water (changing water 3 times a day) for 3 days.

7. Filter the solution and extract it with chloroform (4 times) to remove any free organic material (viz DMAP, DCC, AZT).

8. Freeze dry the aqueous solution.

B. Dextran Sulfate + Succinic Anhydride + AZT Conjugation

General Method

1. Dissolve 50 - 500 mg of dextran sulfate in 10 - 50 ml of dimethyl sulfoxide + pyridine (1:1) mixture by warming.

2. Cool the solution, add 5 - 50 mg of each 4-dimethylamino pyridine (DMAP) and anhydrous sodium sulfate (Na2So4).

3. After 5 - 30 minutes add 50 - 500 mg of succinic anhydride and stir the solution for 1 - 24 hours at room

temperature to 40°C.

4. Add 5 - 50 mg of each 4-dimethyl amino pyridine (DMAP) and dicyclohexl carbodiimide (DCC).

5. Add 50 - 500 mg of AZT and stir the solution at room temperature to 40°C for 12 - 96 hours.

6. Add 25 ml water, cool the solution, filter.

7. Dialyse the solution against water (changing a solution 3 times a day) for 3 days.

8. Filter the solution and extract it with chloroform (4 times) to remove any free organic material (viz DMAP, DCC, AZT).

9. Freeze dry the aqueous solution.

Preferred Method

1. Dissolve 100 mg of dextran sulfate in 25 ml of dimethyl sulfoxide + pyridine (1:1) mixture by warming.

2. Cool the solution, add 20 mg of each 4-dimethylamino pyridine (DMAP) and anhydrous sodium sulfate (Na2So4).

3. After 5 minutes add 200 mg of succinic anhydride and stir the solution for 3 hours at room temperature.

4. Add 20 mg of each 4-dimethyl amino pyridine (DMAP) and dicyclohexl carbodiimide (DCC).

5. Add 100 mg of AZT and stir the solution at room temperature for 72 hours.

6. Add 25 ml water, cool the solution, filter.

7. Dialyse the solution against water (changing a solution 3 times a day) for 3 days.

8. Filter the solution and extract it with chloroform (4 times) to remove any free organic material (viz DMAP, DCC, AZT).

9. Freeze dry the aqueous solution.

C. Dextran-EDTA-Monoanhydride-AZT Conjugation

General Method

1. Dissolve 200 mg - 2 gm of EDTA-monoanhydride in 10 - 50 ml dimethyl sulfoxide (DMSO) by warming, cool and 0.1 - 2 ml triethylamine (TEA).

2. Add 0.2 - 2 gm of dextran, warm the solution to dissolve dextran and stir the reaction mixture at room temperature to 40°C for 2 - 24 hours.

3. Add 5 - 50 mg of each 4-dimethylamino pyridine (DMAP) and dicyclohexyl carbodiimide (DCC) and 50 - 500 mg of AZT.

4. Stir the reaction mixture at room temperature to 40°C for 12 - 96 hours.

5. Add 20 ml water, cool, filter, and dialyse against water for 3 days.

6. Filter the solution and extract it with chloroform (4 times) to remove any free organic material.

7. Freeze dry the aqueous solution.

Preferred Method

1. Dissolve 1 gm of EDTA-monoanhydride in 20 ml dimethyl sulfoxide (DMSO) by warming, cool and 1 ml triethylamine (TEA).

2. Add 1 gm of dextran, warm the solution to dissolve dextran and stir the reaction mixture at room temperature for 6 hours.

3. Add 20 mg of each 4-dimethylamino pyridine (DMAP) and dicyclohexyl carbodiimide (DCC) and 100 mg of AZT.

4. Stir the reaction mixture at room temperature for 72 hours.

5. Add 20 ml water, cool, filter, and dialyse against water for 3 days.

6. Filter the solution and extract it with chloroform (4 times) to remove any free organic material.

7. Freeze dry the aqueous solution.

D. **Dextran-EDTA-Monoanhydride-AZT Conjugation**

General Method

1. Dissolve 10 - 100 mg of EDTA-monoanhydride in 1 - 10 ml dimethyl sulfoxide (DMSO) by warming, cool, add 0.1 - 1 ml of triethylamine and 20 - 100 mg of AZT.

2. Stir the solution at room temperature to 40°C for 2 - 24 hours.

3. Add 5 - 50 mg of each 4-dimethylamino pyridine (DMAP) and dicyclohexyl carbodiimide (DCC) and 25 - 250 mg of AZT. Stir the reaction mixture for 1 - 24 hours.

4. Again add 5 - 50 mg of each 4-dimethyl amino pyridine and dicyclohexyl carbodiimide.

5. Dissolve 50 - 500 mg of dextran in 5 - 25 ml DMSO by warming, cool it, and add this solution to above mixture.

6. Stir the reaction mixture overnight at room temperature to 40°C.

7. Add 20 ml water, cool it, filter and dialyse against water for 3 days.

8. Filter, extract with chloroform (4 times) to remove any organic material.

9. Freeze dry aqueous solution.

Preferred Method

1. Dissolve 28 mg of EDTA-monoanhydride in 2 ml dimethyl sulfoxide (DMSO) by warming, cool, add 0.1 ml of triethylamine and 27 mg of AZT.

2. Stir the solution at room temperature for 6 hours.

3. Add 10 mg of each 4-dimethylamino pyridine (DMAP) and dicyclohexyl carbodiimide (DCC) and 54 mg of AZT. Stir the reaction mixture for 3 hours.

4. Again add 10 mg of each 4-dimethyl amino pyridine and dicyclohexyl carbodiimide.

5. Dissolve 100 mg of dextran in 10 ml DMSO by warming, cool it, and add this solution to above mixture.

6. Stir the reaction mixture overnight at room temperature.

7. Add 20 ml water, cool it, filter and dialyse against water for 3 days.

8. Filter, extract with chloroform (4 times) to remove any organic material.

9. Freeze dry aqueous solution.

E. Dextran-Cis-aconitic Anhydride-AZT Conjugation

General Method

1. To a solution of 25 - 250 mg of AZT, 2 - 25 mg of 4-dimethyl amino pyridine and 5 - 100 mg of anhydrous sodium sulfate in 0.5 - 10 ml pyridine, add 25 - 100 mg of cis-aconitic anhydride.

2. Stir the reaction mixture at room temperature to 40°C for 1 - 24 hours.

3. Dissolve 50 - 500 mg of dextran in 5 - 25 ml of DMSO by warming, cool the solution and add 5 - 50 mg of each 4-dimethyl amino pyridine and dicyclohexyl carbodiimide.

4. Add this solution to the above solution of AZT and stir the reaction mixture for 6 - 72 hours at room temperature to 40°C.

5. Add 20 ml water, cool, filter and dialyse against water for 3 days.

6. Filter, extract with chloroform (4 times) to remove any organic material.

7. Freeze dry aqueous solution.

Preferred Method

1. To a solution of 67 mg of AZT, 8 mg of 4-dimethyl amino pyridine and 25 mg of anhydrous sodium sulfate in 2.5 ml pyridine, add 59 mg of cis-aconitic anhydride.

2. Stir the reaction mixture at room temperature for 3 hours.

3. Dissolve 100 mg of dextran in 10 ml of DMSO by warming, cool the solution and add 20 mg of each 4-dimethyl amino pyridine and dicyclohexyl carbodiimide.

4. Add this solution to the above solution of AZT and stir the reaction mixture for 24 hours at room temperature.

5. Add 20 ml water, cool, filter and dialyse against water for 3 days.

6. Filter, extract with chloroform (4 times) to remove any organic material.

7. Freeze dry aqueous solution.

Further Test Results

DRUG	CONCENTRATION (ug/ml)	# OF SYNCYTIA	PER WELL
AD072193B	100	0	0
AZT-Cis-Aconitic	10	0	0
Anhydride +	1	37	33
Dextran	0.1	63	59
AD702193A	100	0	0
AZT + Succinic	10	24	30
Anhydride +	1	60	61
Dextran	0.1	63	68
AD071593	100	0	0
AZT + Succinic	10	0	2
Anhydride +	1	34	37
Dextran	0.1	65	62
AD072793B	100	0	0
AZT+EDTA-	10	0	0
Monoanhydride +	1	14	10
Dextran	0.1	69	65
AD072393	100	0	0
AZT+EDTA-	10	14	17
Monoanhydride +	1	58	61
Dextran	0.1	63	67
AD072743A	100	0	0
AZT+Succinic	10	10	13
Anhydride + N-	1	56	60
hydroxysuccinimide	0.1	64	66
+ Dextran			

ADS072393A	100	0	0
AZT+Succinic	10	0	0
Anhydride +	1	18	13
Dextran Sulfate	0.1	62	66

CONTROLS	CONCENTRATION (ug)	# OF SYNCYTIA	PER WELL
CELLS (NO VIRUS/ NO DRUG)	0	0	0
VIRUS (NO DURG)	66	67	63
AZT POSITIVE CONTROL	2.7 (10.0uM)	0	0
	0.27 (1.00uM)	0	0
	0.027 (0.10uM)	9	12
	0.0027 (0.01uM)	63	65

COUNTS WERE PERFORMED ON DAY FOUR IN DUPLICATE

CODE:

1. UNINFECTED CELLS WITH DRUG SHOWED SOME CYTOTOXICITY
2. UNINFECTED CELLS WITH DRUG SHOWED MARKED CYTOTOXICITY

CLAIMS:

1. A pharmaceutical preparation comprising a complex of AZT and dextrin.

2. A pharmaceutical preparation comprising a complex of AZT and cellulose.

3. The pharmaceutical preparation of claim 1 or 2 useful in the treatment of viral diseases.

4. The pharmaceutical preparation of claim 1 or 2 useful in the treatment of AIDS.

5. The pharmaceutical preparation of claim 1 or 2 including a pharmaceutically acceptable carrier or diluent.

6. A method of medical treatment comprising the administration of the pharmaceutical preparation of claim 1 or 2, in an appropriate dosage form, quantity, regimen and route of administration.

7. A process for the preparation of a pharmaceutical preparation comprising the complexing of AZT with dextrin or cellulose.

8. A process for the preparation of a complex of AZT and dextran comprising:

a. To a solution of 20 - 200 mg of AZT, 2 - 20 mg of 4-dimethyl amino pyridine (DMAP) and 10 - 100 mg of anhydrous sodium sulfate (NA2So4) in 1 - 10 ml of pyridine add 10 - 100 mg of succinic anhydride.

b. Stir the resulting slurry under closed top at room temperature to 40°C for 1 - 24 hours.

c. Add 50 - 500 mg of dextran solution in 5 - 25 ml of dimethyl sulfoxide (DMSO).

d. Add 5 - 50 mg of each 4-dimethylamino pyridine (DMAP) and dicyclohexyl carbodiimide (DCC), stir the reaction mixture for 12 - 96 hours.

e. Add 20 ml water, cool the solution, filter.

f. Dialyse the solution against water (changing water 3 times a day) for 3 days.

g. Filter the solution and extract it with chloroform (4 times) to remove any free organic material (viz DMAP, DCC, AZT).

h. Freeze dry the aqueous solution.

9. A process for the preparation of a complex of AZT and dextran comprising:

a. To a solution of 54 mg of AZT, 6 mg of 4-dimethyl amino pyridine (DMAP) and 20 mg of anhydrous sodium sulfate (NA2So4) in 2 ml of pyridine add 25 mg of succinic anhydride.

b. Stir the resulting slurry under closed top at room temperature for 3 hours.

c. Add 100 mg of dextran solution in 10 ml of dimethyl sulfoxide (DMSO).

d. Add 20 mg of each 4-dimethylamino pyridine (DMAP) and dicyclohexyl carbodiimide (DCC), stir the reaction mixture for 72 hours.

e. Add 20 ml water, cool the solution, filter.

f. Dialyse the solution against water (changing water 3 times a day) for 3 days.

g. Filter the solution and extract it with chloroform (4 times) to remove any free organic material (viz DMAP, DCC, AZT).

h. Freeze dry the aqueous solution.

10. A process for the preparation of a complex of AZT and dextran sulfate comprising:

a. Dissolve 50 - 500 mg of dextran sulfate in 10 - 50 ml of dimethyl sulfoxide + pyridine (1:1) mixture by warming.

b. Cool the solution, add 5 - 50 mg of each 4-dimethylamino pyridine (DMAP) and anhydrous sodium sulfate (Na2So4).

c. After 5 - 30 minutes add 50 - 500 mg of succinic anhydride and stir the solution for 1 - 24 hours at room temperature to 40°C.

d. Add 5 - 50 mg of each 4-dimethyl amino pyridine (DMAP) and dicyclohexl carbodiimide (DCC).

e. Add 50 - 500 mg of AZT and stir the solution at room temperature to 40°C for 12 - 96 hours.

f. Add 25 ml water, cool the solution, filter.

g. Dialyse the solution against water (changing a solution 3 times a day) for 3 days.

h. Filter the solution and extract it with chloroform (4 times) to remove any free organic material (viz DMAP,

DCC, AZT).

i. Freeze dry the aqueous solution.

11. A process for the preparation of a complex of AZT and dextran sulfate comprising:

a. Dissolve 100 mg of dextran sulfate in 25 ml of dimethyl sulfoxide + pyridine (1:1) mixture by warming.

b. Cool the solution, add 20 mg of each 4-dimethylamino pyridine (DMAP) and anhydrous sodium sulfate (Na2So4).

c. After 5 minutes add 200 mg of succinic anhydride and stir the solution for 3 hours at room temperature.

d. Add 20 mg of each 4-dimethyl amino pyridine (DMAP) and dicyclohexl carbodiimide (DCC).

e. Add 100 mg of AZT and stir the solution at room temperature for 72 hours.

f. Add 25 ml water, cool the solution, filter.

g. Dialyse the solution against water (changing a solution 3 times a day) for 3 days.

h. Filter the solution and extract it with chloroform (4 times) to remove any free organic material (viz DMAP, DCC, AZT).

i. Freeze dry the aqueous solution.

12. A process for the preparation of a complex of AZT and dextran comprising:

a. Dissolve 200 mg - 2 gm of EDTA-monoanhydride in 10 - 50

ml dimethyl sulfoxide (DMSO) by warming, cool and 0.1 - 2 ml triethylamine (TEA).

b. Add 0.2 - 2 gm of dextran, warm the solution to dissolve dextran and stir the reaction mixture at room temperature to 40°C for 2 - 24 hours.

c. Add 5 - 50 mg of each 4-dimethylamino pyridine (DMAP) and dicyclohexyl carbodiimide (DCC) and 50 - 500 mg of AZT.

d. Stir the reaction mixture at room temperature to 40°C for 12 - 96 hours.

e. Add 20 ml water, cool, filter, and dialyse against water for 3 days.

f. Filter the solution and extract it with chloroform (4 times) to remove any free organic material.

g. Freeze dry the aqueous solution.

13. A process for the preparation of a complex of AZT and dextran comprising:

a. Dissolve 1 gm of EDTA-monoanhydride in 20 ml dimethyl sulfoxide (DMSO) by warming, cool and 1 ml triethylamine (TEA).

b. Add 1 gm of dextran, warm the solution to dissolve dextran and stir the reaction mixture at room temperature for 6 hours.

c. Add 20 mg of each 4-dimethylamino pyridine (DMAP) and dicyclohexyl carbodiimide (DCC) and 100 mg of AZT.

d. Stir the reaction mixture at room temperature for 72 hours.

e. Add 20 ml water, cool, filter, and dialyse against water for 3 days.

f. Filter the solution and extract it with chloroform (4 times) to remove any free organic material.

g. Freeze dry the aqueous solution.

14. A process for the preparation of a complex of AZT and dextran comprising:

a. Dissolve 10 - 100 mg of EDTA-monoanhydride in 1 - 10 ml dimethyl sulfoxide (DMSO) by warming, cool, add 0.1 - 1 ml of triethylamine and 20 - 100 mg of AZT.

b. Stir the solution at room temperature to 40°C for 2 - 24 hours.

c. Add 5 - 50 mg of each 4-dimethylamino pyridine (DMAP) and dicyclohexyl carbodiimide (DCC) and 25 - 250 mg of AZT. Stir the reaction mixture for 1 - 24 hours.

d. Again add 5 - 50 mg of each 4-dimethyl amino pyridine and dicyclohexyl carbodiimide.

e. Dissolve 50 - 500 mg of dextran in 5 - 25 ml DMSO by warming, cool it, and add this solution to above mixture.

f. Stir the reaction mixture overnight at room temperature to 40°C.

g. Add 20 ml water, cool it, filter and dialyse against water for 3 days.

h. Filter, extract with chloroform (4 times) to remove any organic material.

i. Freeze dry aqueous solution.

15. A process for the preparation of a complex of AZT and dextran comprising:

a. Dissolve 28 mg of EDTA-monoanhydride in 2 ml dimethyl sulfoxide (DMSO) by warming, cool, add 0.1 ml of triethylamine and 27 mg of AZT.

b. Stir the solution at room temperature for 6 hours.

c. Add 10 mg of each 4-dimethylamino pyridine (DMAP) and dicyclohexyl carbodiimide (DCC) and 54 mg of AZT. Stir the reaction mixture for 3 hours.

d. Again add 10 mg of each 4-dimethyl amino pyridine and dicyclohexyl carbodiimide.

e. Dissolve 100 mg of dextran in 10 ml DMSO by warming, cool it, and add this solution to above mixture.

f. Stir the reaction mixture overnight at room temperature.

g. Add 20 ml water, cool it, filter and dialyse against water for 3 days.

h. Filter, extract with chloroform (4 times) to remove any organic material.

i. Freeze dry aqueous solution.

16. A process for the preparation of a complex of AZT and dextran comprising:

a. To a solution of 25 - 250 mg of AZT, 2 - 25 mg of 4-dimethyl amino pyridine and 5 - 100 mg of anhydrous

sodium sulfate in 0.5 - 10 ml pyridine, add 25 - 100 mg of cis-aconitic anhydride.

b. Stir the reaction mixture at room temperature to 40°C for 1 - 24 hours.

c. Dissolve 50 - 500 mg of dextran in 5 - 25 ml of DMSO by warming, cool the solution and add 5 - 50 mg of each 4-dimethyl amino pyridine and dicyclohexyl carbodiimide.

d. Add this solution to the above solution of AZT and stir the reaction mixture for 6 - 72 hours at room temperature to 40°C.

e. Add 20 ml water, cool, filter and dialyse against water for 3 days.

f. Filter, extract with chloroform (4 times) to remove any organic material.

g. Freeze dry aqueous solution.

17. A process for the preparation of a complex of AZT and dextran comprising:

a. To a solution of 67 mg of AZT, 8 mg of 4-dimethyl amino pyridine and 25 mg of anhydrous sodium sulfate in 2.9 ml pyridine, add 59 mg of cis-aconitic anhydride.

b. Stir the reaction mixture at room temperature for 3 hours.

c. Dissolve 100 mg of dextran in 10 ml of DMSO by warming, cool the solution and add 20 mg of each 4-dimethyl amino pyridine and dicyclohexyl carbodiimide.

d. Add this solution to the above solution of AZT and stir

the reaction mixture for 24 hours at room temperature.

e. Add 20 ml water, cool, filter and dialyse against water for 3 days.

f. Filter, extract with chloroform (4 times) to remove any organic material.

g. Freeze dry aqueous solution.

INTERNATIONAL SEARCH REPORT

Intr onal Application No
PCT/CA 94/00449

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,93 11763 (USHER THOMAS, C.) 24 June 1993 see page 11; claims	1-17
P,X	EP,A,0 579 435 (LOFTSSON THORSTEINN) 19 January 1994 see claims 1,16,20	1,3-7
A	EP,A,0 369 463 (EISAI CO.) 23 May 1990 see claims 1,3; examples	8-17
X	EP,A,0 335 545 (UNIVERSITY OF FLORIDA) 4 October 1989 see claims 1,23	1,3-7

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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PCT/CA 94/00449

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